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(57) Abstract

An immunoisolatory vehicle for the implantation into an individual of cells which produce a needed product or provide a needed metabolic function. The vehicle is comprised of a core region containing isolated cells and materials sufficient to maintain the cells, and a permselective, biocompatible, peripheral region free of the isolated cells, which immunoisolates the core yet provides for the delivery of the secreted product or metabolic function to the individual. The vehicle is particularly well-suited to delivery of insulin from immunoisolated islets of Langerhans, and can also be used advantageously for delivery of high molecular weight products, such as products larger than IgG. A method of making a biocompatible, immunoisolatory implantable vehicle, consisting in a first embodiment of a coextrusion process, and in a second embodiment of a stepwise process. A method for isolating cells within a biocompatible, immunoisolatory implantable vehicle, which protects the isolated cells from attack by the immune system of an individual in whom the vehicle is implanted. A method of providing a needed biological product or metabolic function to an individual, comprising implanting into the individual an immunoisolatory vehicle containing isolated cells which produce the product or provide the metabolic function.

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IMPLANTABLE BIOCOMPATIBLE IMMUNOISOLATORY VEHICLE FOR DELIVERY OF SELECTED THERAPEUTIC PRODUCTS

Related Application

This is a continuation-in-part application under 35 USC 120 of application U.S.S.N. 07/692,403, filed 25 April 1991.

Background

Many clinical conditions, deficiencies, and disease states can be remedied or alleviated by supplying to the patient a factor or factors produced by living cells or removing from the patient deleterious factors which are metabolized by living cells. In many cases, these factors can restore or compensate for the impairment or loss of organ or tissue function. Examples of disease or deficiency states whose etiologies include loss of secretory organ or tissue function include (a) diabetes, wherein the production of insulin by pancreatic islets of Langerhans is impaired or lost; (b) hypoparathyroidism, wherein the loss of production of parathydroid hormone causes serum calcium levels to drop, resulting in severe muscular tetany; (c) Parkinsonism, wherein dopamine production is diminished; and (d) anemia, which is characterized by the loss of production of red blood cells secondary to a deficiency in erythropoetin. The impairment or loss of organ or tissue function may result in the loss of additional metabolic functions. For example, in fulminant hepatic failure, liver tissue is rendered incapable of removing toxins, excreting the products of cell metabolism, and secreting

ess ntial products, such as albumin and Factor VIII. Bontempo, F.A., et al., (1987) Blood 69:1721-1724.

In other cases, these factors are biological response modifiers, such as lymphokines or cytokines, which enhance the patient's immune system or act as anti-inflammatory agents. These can be particularly useful in individuals with a chronic parasitic or infectious disease, and may also be useful for the treatment of certain cancers. It may also be desirable to supply trophic factors to a patient, such as nerve growth factor or insulin-like growth factor-one or -two (IGF1 or IGF2).

In many disease or deficiency states, the affected organ or tissue is one which normally functions in a manner responsive to fluctuations in the levels of specific metabolites, thereby maintaining homeostasis. For example, the parathyroid gland normally modulates production of parathyroid hormone (PTH) in response to fluctuations in serum calcium. Similarly, β cells in the pancreatic islets of Langerhans normally modulate production of insulin in response to fluctuations in serum glucose. Traditional therapeutic approaches to the treatment of such diseases cannot compensate for the responsiveness of the normal tissue to these fluctuations. For example, an accepted treatment for diabetes includes daily injections of insulin. This regimen cannot compensate for the rapid, transient fluctuations in serum glucose levels produced by, for example, strenuous exercise. Failure to provide such compensation may lead to complications of the disease state; this is particularly true in diabetes. Jarret, R.J. and Keen J., (1976) Lancet(2):1009-1012.

Accordingly, many investigators have attempted to reconstitute organ or tissue function by transplanting whole organs, organ tissue, or cells which provide

secreted products or affect metabolic functions. Moreover, transplantation can provide dramatic benefits but is limited in its application by the relatively small number of organs suitable and available for grafting. In general, the patient must be immunosuppressed in order to avert immunological rejection of the transplant, which results in loss of transplant function and eventual necrosis of the transplanted tissue or cells. In many cases, the transplant must remain functional for a long period of time, even for the remainder of the patient's lifetime. It is both undesirable and expensive to maintain a patient in an immunosuppressed state for a substantial period of time.

A desirable alternative to such transplantation procedures is the implantation of cells or tissues within a physical barrier which will allow diffusion of nutrients, waste materials, and secreted products, but block the cellular and molecular effectors of immunological rejection. A variety of devices which protect tissues or cells producing a selected product from the immune system have been explored. These include extravascular diffusion chambers, intravascular diffusion chambers, intravascular ultrafiltration chambers, and implantation of microencapsulated cells. Scharp, D.W., et al. (1984) World J. Surg. 8:221-9. These devices were envisioned as providing a significant advance in the field of transplantation, as they would alleviate the need to maintain the patient in an immunosuppressed state, and would thereby allow many more patients to recieve restorative or otherwise beneficial transplants by allowing the use of donor cells or tissue which could not have been used with the conventional transplantation techniques. However, none of these approaches have been satisfactory for providing long-term transplant function. A method of delivering appropriate quantities of needed substances, such as enzymes and hormones, or of providing

other needed metabolic functions, for an extended p riod of time is still unavailable and would be very advantageous to those in need of long-term treatment.

Summary of the Invention

This invention relates to a biocompatible, immunoisolatory, implantable vehicle. The instant vehicle is suitable for isolating biologically active cells or substances from the body's protective mechanisms following implantation into an individual. The instant vehicle is comprised of (a) a core which contains isolated cells, either suspended in a liquid medium or immobilized within a hydrogel matrix, and (b) a surrounding or peripheral region ("jacket") of permselective matrix or membrane which does not contain isolated cells, which is biocompatible, and which is sufficient to protect the isolated cells in the core from immunological attack.

Herein, the term "individual" refers to a human or an animal subject.

As defined herein, the term "dual matrix vehicles" refers to such vehicles with a cell-containing core and an external jacket free of cells. In one embodiment, the matrix core is formed of a hydrogel which is cross-linked to a hydrogel jacket, suitably in the form of a rod or other shape. The hydrogel jacket may be formed independently as a sheath around the matrix without cross-linking. The hydrogel care is not necessarily linked to the outer jacket by means of opposite ionic charges. In another embodiment, the external jacket is formed of a thermoplastic material which is not linked to the core matrix by chemical bonding.

As used herein, unless otherwise specified, the term "cells" means cells in any form, including but not limited

to cells retained in tissue, cell clusters, and individually isolated cells, unless otherwise specified.

The core of the immunoisolatory vehicle is constructed to provide a suitable local environment for the particular cells isolated therein. In some embodiments, the core comprises a liquid medium sufficient to maintain the cells. Liquid cores are particularly suitable for maintaining transformed cells, such as PC12 cells. In other embodiments, the core comprises a gel matrix which immobilizes and distributes the cells, thereby reducing the formation of dense cellular agglomerations. The gel matrix may be composed of hydrogel or extracellular matrix components.

Cores made of a hydrogel matrix are particularly suitable for maintaining cells or tissues which tend to form agglomerates, such as the cells in islets of Langerhans, or adrenal chromaffin cells. The matrix should be of sufficient viscosity to maintain cell dispersion within the matrix. Optionally, the core of the instant vehicle can contain substances which support or promote the function of the isolated cells. These substances include natural or synthetic nutrient sources, extracellular matrix (ECM) components, growth factors or growth regulatory substances, or a population of feeder or accessory cells or O_2 carriers such as hemoglobins and fluorocarbons.

In previously existing devices, the core and jacket were linked via ionic bonds between oppositely charged polymers in one of two ways. (1) The devices of Rha (U.S. Patent No; 4,744, 933) were constructed of a charged inner core material and an outer jacket material of the opposite charge. (2) The devices of Lim and Sun (U.S. Patent Nos: 4,352,833 and 4,409,331) included an intermediate layer of p ly-L-lysine (PLL), which is positively charged, to link

the negatively charged core with the negatively charged jacket material. The elimination of a PLL layer is advantageous in that PLL is believed to be fibrogenic in the host. PLL may also have unwanted growth effects for some cells. Also, the jacket of the device of the invention can be controlled for permselectivity better than those made with PLL.

The jacket of the instant vehicle is made of a material which may be the same as that of the core or may be different. In either case, the material used results in a surrounding or peripheral region which is permselective, biocompatible and immunoisolatory.
The jacket may be formed freely around the core without chemical bonding or, alternatively, the jacket may be directly cross-linked to the core matrix. In either case, formation of the vehicle of the present invention does not require polymers of opposite charge to the core being present in an interfacial layer or in the jacket.

Preferably, the core and external jacket form an interface free of "ionic bonding" between oppositely charged polymers and free of an intermediate layer of the type used in prior art microcapsules. Ionic bonding refers to an ionic interaction of a core of one charge (positive or negative) and the jacket (or an intermediate layer) of opposite charge.

The jacket allows passage of substances up to a predetermined size, but prevents the passage of larger substances. More specifically, the surrounding or peripheral region is produced in such a manner that it has pores or voids of a predetermined range of sizes; as a result, the vehicle is permselective. The molecular weight cutoff (MWCO) selected for a particular vehicle will be determined in part by the type and extent of immunological rejection it is anticipated will be

encountered after the vehicl is implanted and in part by the molecular size of the largest substance to be allowed to pass into and/or out of the vehicle. For example, materials can be used to form permselective membranes or hydrogel matrices which allow passage of molecules up to about the size of C1q, a component of complement (about 400 kD), a protein required for the assembly of the cytolytic complement attack complex. In this instance, substances smaller than C1q can pass freely. It is also possible to form permselective matrices or membranes which allow passage of molecules up to about the size of immunoglobulin G (about 150 kD) and exclude larger molecules. Further, membranes or hydrogels which allow passage of molecules up to about the size of immunoglobulin M (about 1,000 kD) can be used; only very large substances, such as cells, will be excluded in this embodiment.

The jacket is also biocompatible. That is, it does not elicit a detrimental host response sufficient to result in rejection of the implanted vehicle or to render it inoperable. Neither does the jacket elicit unfavorable tissue responses such as fibrosis. In addition, the external surface can be selected or designed in such a manner that it is particularly suitable for implantation at the selected site. For example, the external surface can be smooth, stippled or rough, depending on whether attachment by cells of the surrounding tissue is desirable. The shape or configuration can also be selected or designed to be particularly appropriate for the implantation site chosen.

The jacket of the present vehicle is further immunoisolatory. That is, it protects cells in the core of the vehicle from the immune system of the individual in whom the vehicle is implanted. It does so (1) by preventing harmful substances f the individual's body

from entering the core of the vehicle, (2) by minimizing contact between the individual and inflammatory, antigenic, or otherwise harmful materials which may be present in the core and (3) by providing a spatial barrier sufficient to prevent immunological contact between the isolated cells and detrimental portions of the individual's immune system. The vehicle of the present invention is distinguished from the microcapsules of Lim and Sun (Lim, F., Sun, A.M., Science 210:908-910 [1980]; Sun, A.M., Methods in Enzymology 137: 575-579 [1988]) by its outer jacket which ensures that cells cannot project outside of the core. The capsules of Lim and Sun suffered from the disadvantage that portions of encapsulated cells could potentially project from the core through the poly-L-lysine layer and thereby be more likely to elicit inflammatory responses from the host's immune system. This microcapsule technology relies on the presence of potentially bioactive ionic bonds to form the microcapsule. By virtue of their ionic nature, these microcapsules are susceptible to deterioration following implantation due to competition for the ionic bonds that take place in the body of the host after capsule implantation. This problem is minimized by the relatively non-ionic macrocapsules of the present invention. A further advantage of the macrocapsules of the present invention lies in their capacity to contain more cells in a single device than is possible in microcapsule technology.

The surrounding or peripheral region (jacket) can be made of a hydrogel matrix or of a different material, such as a thermoplastic membrane. It can also be made of a matrix-membrane composite, such that a permselective thermoplastic membrane having matrix-filled pores, is formed.

Suitably, the external jacket may b formed f a thermoplastic material known to be biocompatible, such as the ones described herein. In addition, other jackets which have been used in the microcapsule field may also be used herein, such as alginate, suitably cross-linked with a multivalent ion such as calcium.

The immunoisolatory vehicle is useful (a) to deliver a wide range of cellular products, including high molecular weight products, to an individual in need of them, and/or (b) to provide needed metabolic functions to an individual, such as the removal of harmful substances. The instant vehicle contains a multiplicity of cells, such that implantation of a few or a single vehicle is sufficient to provide an effective amount of the needed substance or function to an individual. A further advantage offered by the instant vehicle is practicality of retrieval.

In one embodiment of the preferred invention, which is particulary useful with islets of Langerhans, both the core and the surrounding or peripheral region of the instant vehicle are hydrogels, which can be the same composition hydrogel or different composition hydrogels.

This invention relates further to a method of making a biocompatible immunoisolatory vehicle. In a first embodiment, the vehicle is formed by coextruding from a nested-bore extrusion nozzle materials which form the core and surrounding or peripheral regions, under conditions sufficient to gel, harden, or cast the matrix or membrane precursor(s) of the surrounding or peripheral region (and of the core region). A particular advantage of this coextrusion embodiment is that the cells in the core are isolated from the moment of formation of the vehicle, ensuring that the core materials do not become contaminated or adulterated during handling of the vehicle

pri r to implantation. A furth r advantage of the coextrusion process is that it ensures that the surrounding or peripheral region is free of cells and other core materials. The permeability and biocompatibility characteristics of the surrounding or peripheral region are determined by both the matrix or membrane precursor materials used, and the conditions under which the matrix or membrane is formed.

In a second embodiment of the present method, the immunoisolatory vehicle is formed stepwise. For example, if the immunoisolatory vehicle being made includes a hydrogel core containing the isolated cells, the core can be formed initially, and the surrounding or peripheral matrix or membrane can be assembled or applied subsequently. Conversely, the surrounding or peripheral matrix or membrane can be preformed, and then filled with the preformed isolated-cell containing core material or with materials which will form the core (i.e., core presursor materials). The vehicle is sealed in such a manner that the core materials are completely enclosed. If a core precursor material is used, the vehicle is then exposed to conditions which result in formation of the core.

This invention relates also to a method of delivering a biological product or altering a metabolic or immunologic function in an individual in need of the biological product or altered metabolic function. An immunoisolatory vehicle of the present invention is implanted into the individual (referred to as the recipient), using known techniques or methods and selected for the particular immunoisolatory vehicle and site of implantation. Once implanted, cells isolated within the biocompatible immunoisolatory vehicle produce the desired product(s) or perform the desired function(s). If products are released by the isolated cells, they pass through the surrounding

or p ripheral permselective membrane or hydrogel matrix into the recipient's body. If metabolic functions are provided by the isolated cells, the substances to be metabolized (e.g., degraded or inactivated) enter the vehicle from the recipient's body and are removed from the recipient's bloodstream.

Thus, this invention further relates to a method of isolating cells within a biocompatible, immunoisolatory implantable vehicle, thereby protecting the cells within the vehicle from immunological attack after being implanted into an individual. Although some low molecular weight mediators of the immune responses (e.g. cytokines) may be permeable to the membrane, in most cases local or circulating levels of these substances are not high enough to have detrimental effects. The isolated cells are protected from attack by the recipient's immune system and from potentially deleterious inflammatory responses from the tissues which surround the implanted vehicle. In the core of the vehicle, the isolated cells are maintained in a suitable local environment. In this manner, needed substances or metabolic functions can be delivered to the recipient even for extended periods of time, and without the need to treat the recipient with dangerous immunosuppresive drugs.

Brief Description of the Figures

Figure 1 is a graphic representation of the differences in the permeability of alginate matrices formed under different conditions to test solutes of various molecular sizes.

Figure 2 is a graphic representation of the results of a perifusion test of the functionality of immunoisolated *versus* unprotected islets maintained *in vitro* for four weeks. Figure 2A depicts the results obtained with an immunoisolatory vehicle having a hydrogel core matrix and a peripheral jacket made of a permselective thermoplastic

membrane. Figure 2B depicts the results obtained with an immunois latory vehicle having a hydrogel c r matrix and a peripheral hydrogel jacket.

Figure 3 is a graphic representation showing the total amount of insulin released, and the amounts released during the first and second phase responses in the perifusion test also shown in Figure 2. Figure 3A depicts the results obtained with the dual-matrix immunoisolatory vehicle and Figure 3B depicts the results obtained with the core matrix-permselective membrane immunoisolatory vehicle.

Figure 4 is a graphic representation of the decrease in plasma glucose levels observed when immunoisolated xenogeneic islets are implanted into streptozotocin-induced diabetic mice for a period of 60 days. The immunoisolatory vehicle used was of the configuration described in Example 5.

Figure 5 is a graphic representation of insulin release in a perifusion experiment using an immunoisolatory vehicle containing rat islets, of the configuration described in Example 5, recovered after a period of residence *in vivo* and challenged with glucose, with and without theophylline stimulation.

Figure 6 is a graphic representation of the decrease in plasma glucose levels observed when immunoisolated xenogeneic islets are implanted into streptozotocin-induced diabetic mice for a period of 100 days. The immunoisolatory vehicle used was of the configuration described in Example 4.

Figure 7 is a graphic representation of the permeability of an alginate matrix to various test solutes. Permeabilities were tested after storage in Hank's

solution after 16 hours and 160 hours. The change in permeability is due to leaching of Ca^{++} from the matrix.

Figure 8 is a graphic comparison of the response to glucose challenge of rat islets isolated within dual matrix immunoisolatory vehicles with either thermoplastic or alginate jackets, following a period of residence *in vivo* in discordant xenogeneic recipients (guinea pigs).

Figure 9 is a graphic representation of the partial restoration of normal motor behavior to rodents with experimentally induced Parkinson-like behavior, following implantation of an immunoisolatory vehicle containing adrenal chromaffin cells in a core matrix, with a surrounding jacket of permselective thermoplastic membrane.

Figure 10 is a graphic representation of the mean body weight changes seen in quinolinic acid lesioned rats. Rats receiving immunoisolatory capsules containing bovine adrenal chromaffin cells maintained body weight significantly better than the other lesioned rats.

Figure 11 is a graphic representation of the nonfasting plasma glucose concentrations of diabetic mice after implantation of type 2 acrylic copolymer hollow fibers containing either 1000 rat islets (A) or 500 rat islets (B) implanted either intraperitoneally (circles) or subcutaneously (squares).

Figure 12 is a graphic representation of the effects on blood glucose in diabetic rats implanted with rat islets encapsulated in flat sheet devices.

Detailed Description of the Invention

This invention relates to a bi c mpatible immun isolatory vehicle suitable for long-term implantation into

individuals. Because of its bi compatibility, th vehicle is suitable for long-term isolati n of biologically useful cells and/or substances from th various protectiv systems of the body. As used herein, the term "protective systems" refers to the types of immunological attack which can be mounted by the immune system of an individual in whom the instant vehicle is implanted, and to other rejection mechanisms, such as the fibrotic response, foreign body résponse and other types of inflammatory response which can be induced by the presence of a foreign object in the individuals' body. Implants of the vehicle and contents thereof as described by the current invention retain functionality for greater than three months *in vivo* and in many cases for longer than a year. In addition, the vehicle of the current invention may be prepared of sufficient size to deliver an entire therapeutic dose of a substance from a single or just a few (less than 10) implanted and easily retrievable vehicles.

As used herein, the term "biocompatible" refers collectively to both the intact vehicle and its contents. Specifically, it refers to the capability of the implanted intact vehicle and its contents to avoid detrimental effects of the body's various protective systems and remain functional for a significant period of time. In addition to the avoidance of protective responses from the immune system, or foreign body fibrotic response, "biocompatible" also implies that no specific undesirable cytotoxic or systemic effects are caused by the vehicle and its contents such as would interfere with the desired functioning of the vehicle or its contents.

Important for biocompatibility and continued functionality are vehicle morphology, hydrophobicity and the absence of undesirable substances either on the surface of, or leachable from, the vehicle itself. Thus, brush surfaces, folds, interlayers or other shapes or structures eliciting

a foreign body response are avoided. The vehicl -forming materials are sufficiently pure that unwanted substances do not leach out from the vehicle materials themselves. Additionally, following vehicle preparation, the treatment of the external surface of the vehicle with fluids or materials (e.g. serum) which may adhere to or be absorbed by the vehicle and subsequently impair vehicle biocompatibility are avoided.

This invention also pertains to a method of isolating or protecting implanted cells, tissues, or other materials from immunological attack. The method and vehicle of the instant invention are useful to deliver a wide range of cellular products, including high molecular weight products, to an individual in need of them, and/or to provide needed metabolic functions to an individual, such as the removal of harmful substances. Products which can be delivered using the instant vehicle include a wide variety of factors normally secreted by various organs or tissues. For example, insulin can be delivered to a diabetic patient, dopamine to a patient suffering from Parkinson's disease, or Factor VIII to a Type A hemophiliac. Other products which can be delivered through use of the instant vehicle include trophic factors such as erythropoeitin, growth hormone, Substance P, and neurotensin. Another family of products suited to delivery by the instant vehicle comprises biological response modifiers, including lymphokines and cytokines. Antibodies from antibody secreting cells may also be delivered. Specific antibodies which may be useful include those towards tumor specific antigens. The release of antibodies may also be useful in decreasing circulating levels of compounds such as hormones or growth factors. These products are useful in the treatment of a wide variety of diseases, inflammatory conditions or dis rders, and cancers. The instant vehicle can also be used to restore r augment vital metabolic functions, such

as the removal of toxins or harmful metabolites (e.g., cholesterol) from the bloodstream by cells such as hepatocytes. The method and vehicle of the instant invention make possible the implantation of cells without the concomitant need to immunosuppress the recipient for the duration of treatment. Through use of the biocompatible immunoisolatory vehicle, homeostasis of particular substances can be restored and maintained for extended periods of time. The instant vehicle may contain a multiplicity of cells, such that implantation of a single vehicle can be sufficient to provide an effective amount of the needed substance or function to an individual.

The biocompatible immunoisolatory vehicle of the instant invention comprises (a) a core which contains a biologically active moiety, either suspended in a liquid medium or immobilized within a hydrogel or extracellular matrix, and (b) a surrounding or peripheral region of permselective matrix or membrane (jacket) which does not contain isolated cells, which is biocompatible, and which is sufficient to protect isolated cells if present in the core from immunological attack.

For purposes of the present invention, a biologically active moiety is a tissue, cell, or other substance, which is capable of exerting a biologically useful effect upon the body of an individual in whom a vehicle of the present invention containing a biologically active moiety is implanted. Thus, the term "biologically active moiety" encompasses cells or tissues which secrete or release a biologically active solute; cells or tissues which provide a metabolic capability or function, such as the removal of specific solutes from the bloodstream; or a biologically active substance such as an enzyme, trophic factor, hormone, or biological response modifier.

When the biologically activ moiety within the c re of th biocompatible immunoisolatory vehicle comprises cells, the core is constructed to provide a suitable local environment for the continued viability and function of the cells isolated therein. The instant vehicle can be used to immunoisolate a wide variety of cells or tissues, spanning the range from fully-differentiated, anchorage-dependent cells or primary tissues, through incompletely-differentiated fetal or neonatal tissues, to anchorage-independent transformed cells or cell lines.

Many transformed cells or cell lines are most advantageously isolated within a vehicle having a liquid core. For example, PC12 cells (which secrete dopamine and are herein shown to be useful for the treatment of Parkinsonism) can be isolated within a vehicle whose core comprises a nutrient medium, optionally containing a liquid source of additional factors to sustain cell viability and function, such as fetal bovine or equine serum.

Suitably, the core may be composed of a matrix formed by a hydrogel which stabilizes the position of the cells in cell clumps. The term "hydrogel" herein refers to a three dimensional network of cross-linked hydrophilic polymers. The network is in the form of a gel substantially composed of water, preferably but not limited to gels being greater than 90% water. Cross-linked hydrogels can also be considered solids because they do not flow or deform without appreciable applied shear stress.

Compositions which form hydrogels fall into two classes for the purposes of this application. The first class carries a net negative charge and is typified by alginate. The second class carries a net postive charge and is typified by extracellular matrix components such as c llagen and laminin. Examples of commercially available

extracellular matrix components include Matrigel™ and Vitrogen™.

By way of example, cells which do not require an anchorage substrate are those which are able to form clumps or agglomerates and thus provide anchorage for each other. Exemplary clumping cell types are pancreatic islets, pancreatic beta-cell lines, Chinese hamster ovary (CHO) cells, and adrenal chromaffin cells. These cells are suitably enclosed in a negatively charged matrix such as alginate.

Fibroblasts generally survive well in a positively charged matrix and are thus suitably enclosed in extracellular-matrix type hydrogels. Certain cell types tend to multiply rapidly and could overgrow the space available within the core if they do not exhibit growth arrest. If the isolated cells do not exhibit growth arrest upon confluence, substances which induce quiescence can be included in the interior of the vehicle. In some instances, a hydrogel core may suffice to limit continued proliferation. For example, a hydrogel matrix precursor solution can be included but not exposed to polymerizing conditions. In the case of sodium alginate, a hydrogel will form slowly after implantation as calcium ions are scavenged from the surrounding tissues. Alternatively, growth inhibitory factors, or stimulators of differentiation can be incorporated into microbeads of a slowly degraded polymer such as polycarbonate, and cosuspended with the product-secreting cells. For instance, NGF or FGF can be used to stimulate PC-12 cell differentiation and terminate cell division.

Other cells, particularly primary cells or tissues, tend to adhere to each other and form dense agglomerations which develop central necrotic regions due to the relative inaccessibility of nutrients and oxygen to cells embedded

within the agglomerated masses. These dense cellular masses can form slowly, as a result of cell growth into dense colonies, or rapidly, upon the reassociation of freshly-dispersed cells or tissue mediated by cell-surface adhesion proteins. Cells or tissues which are highly metabolically active are particularly susceptible to the effects of oxygen or nutrient deprivation, and die shortly after becoming embedded in the center of an agglomerate. Many endocrine tissues, which normally are sustained by dense capillary beds, exhibit this behavior; islets of Langerhans and adrenal chromaffin cells are particularly sensitive. Cells or tissues which exhibit this behavior perform most satisfactorily in vehicles comprising a hydrogel matrix core, sufficient to immobilize the cells or tissue, thereby preserving the access of nutrients and oxygen to the majority of them. In other circumstances, the immobilizing hydrogel matrix further performs the additional function of producing or preserving functional units of a size and/or shape appropriate for maintaining desirable characteristics of the isolated cells. Moreover, the presence of the core matrix allows maintenance of a uniform distribution of cells or clusters of cells within the vehicle (i.e., the core matrix prevents settling and decreses mobility of the included cells).

One particularly advantageous use of hydrogel cores pertains to the encapsulation of actively dividing cells. Alginate or other hydrogels may be included in suspensions of actively dividing cells to be encapsulated. Following encapsulation and generation of the gel, the encapsulated cells are somewhat immobilized within the gel and new cells produced during cell division stay localized near the parent cell. In this manner clusters of cells are produced within the core. Such a growth method is advantageous in the case of cells such as the beta cell derived NIT cell line. In the absence of a core matrix

these cells tend to gr w attached along the inner walls f the encapsulation device, with very few cells growing feely within the cavity of the device. Growth only on the walls of the capsule leads to a cell population size that is restricted by the surface area of the inner capsule wall as opposed to a population that grows to fill the vehicle cavity. When alginate is included in the core, cell growth is no longer limited to the inner capsule surface. Rather, discrete spheres of NIT cells are produced throughout the core, resulting in a significantly larger total cell population than that which occurs in the absence of alginate.

Even in the presence of a core matrix, the size of tissue fragments which can be loaded into a given vehicle volume is limited by the appearance of central necrosis within the individual fragments. In one aspect of the instant invention, the useful amount of tissue fragments or cell clusters that can be placed within the immunoisolatory vehicle is increased by preparing the cells in a form with improved diffusional characteristics. Generally this means preparation of the tissue fragments to a size less than 75μm diameter and most optimally to about 35μm diameter or less for vehicles to be implanted peritoneally. Aggregates or clusters of cells in improved diffusional form are prepared for spontaneously reaggregating cells (e.g. pancreatic islets or adrenal chromaffin cells) by enzymatically dispersing the tissue, to single cell and small cell aggregate suspensions, followed by controlled reaggregation to the improved diffusional form.

Herein, the term "aggregating" refers to a process of promoting cell clustering. The cells which form clusters may be obtained from naturally occurring agglomerates, such as pancreatic islets which are dispersed into single or small-clump suspension and subsequently reaggr gated

according t the methods described. Alternatively, the cells may be obtained originally as single cells or small cell clumps, and then aggregated to form the desired cluster size. Such cell clusters will generally contain about 3 - 400 cells, depending upon cell size and aggregation characteristics. Typically, the cluster contains about 10 to about 50 cells. The use of reaggregated pancreatic islet cells is advantageous for insuring proper diffusion characteristics within the core and maintaining islet viability. Reaggregated islets also allow the use of smaller capsules. For instance, 500 non-reaggregated islets would generally require a capsule of approximately 14 cm in length (2% density). In contrast, capsules containing islets reaggregated to a size smaller than an intact islet may be as small as 1 to 2 cm in length because of more efficient packing. More efficient packing allows a lower pO_2 outside the fiber to be tolerated without resultant necrotic cores. Built in tolerance for lower outside pO_2 has at least two advantages. Firstly, a smaller capsule size may be used to contain the same number of cells, i.e. increased tissue density within the implant is better tolerated. Secondly, implantation sites with known reduced pO_2, such as subcutaneous locations, may be used successfully. The presence of the alginate matrix further insures that the aggregates will not reassociate to large cell masses where internal cells would be deprived of nutrients and/or oxygen.

Pancreatic islet cells still retain functionality and secrete insulin in response to glucose in near normal fashion following enzymatic dispersion and reaggregation. Cells from dispersed islets are reaggregated to the desired cluster size prior to loading into the vehicle. Reaggregation can be accomplished by the methods described by Britt, Diabetes 34: 898-903, or by similar m thods. The optimal aggregate size for islets is the smallest size

cluster which still maintains the desired physiological characteristics. The matrix or matrix forming materials may then be added to the cells, and the c mbinati n may be coextruded into or loaded into biocompatible immunoisolatory vehicles. If necessary, matrix formation may then be induced. In preferred embodiments, cells are reaggregated overnight at 37°C, without agitation. The development of aggregates is monitored by light microscopy until aggregates achieve a size of 25 to 75μm preferably 35μm diameter. Liquid uncrosslinked alginate is then added to the cells to a concentration of from 0.5 to 2%, the cells are incorporated into vehicles, the vehicles are sealed if necessary, and polymerization is induced by immersion of the vehicle in $CaCl_2$.

Primary cells or tissues may be useful with the vehicle of the instant invention for various medical applications. For regulatory reasons and reasons of patient safety, it may sometimes be useful to employ as sources for primary cultures, animals of carefully controlled hereditary and developmental background. The presence of unwanted virus, bacteria and other pathogens may be limited through the use of specific pathogen free or gnotobiotic source animals. References and methods for the establishment, care and use of specific pathogen free and gnotobiotic herds are provided by Maniats, O.P., et al. (1978) Can. J. Med.42:428, Matthews P.J., et al., (1981) Recent Advances in Germ Free Research 61-64, Tokai Univ. Press, and in the National Accreditation Standards publication of the National SPF Swine Accrediting Agency, Inc., Conrad, Iowa.

Optionally, a matrix core can also contain materials which support or promote the function of the isolated cells. For example, extracellular matrix (ECM) components can be included to promote specific attachment or adhesion of the isolated cells. A combination of ECM components which is particularly suitable for fostering the growth of certain

types of cells is taught in Kleinman et al., (1989) U.S. Patent No. 4,829,000.The core matrix can provide a reservoir for soluble or releasable substances, such as growth factors or growth regulatory substances, or for natural or synthetic substances which enhance or improve the supply of nutrients or oxygen to the isolated cells. Thus, it can function in a manner similar to the bone marrow ECM, which has been reported to behave as a slow-release reservoir for myeloid lineage-specific growth factors such as granulocyte-macrophage colony stimulating factor (gmcsf). Gordon, M.Y., et al., (1987) Nature 326:403-405. Thus, the core matrix can function as a reservoir for growth factors (e.g., prolactin, or insulin-like growth factor 2), growth regulatory substances such as transforming growth factor β (TGFβ) or the retinoblastoma gene protein or nutrient-transport enhancers (e.g., perfluorocarbons, which can enhance the concentration of dissolved oxygen in the core). Certain of these substances are also appropriate for inclusion in liquid media.

Additionally, a population of feeder or accessory cells can be coisolated within the vehicle. For example, hepatocytes can be coisolated with endothelial accessory cells, Cai, Z., et al., (1988) Artificial Organs, 12(5):388-393, or mixed with islet cells, Ricordi C., et al., (1987) Transplantation 45(6):1148-1151, or adrenal chromaffin cells can be coisolated with accessory cells which provide nerve growth factor (NGF), a substance needed by the chromaffin cells. In the latter case, fibroblasts which have been transfected with an expression vector for NGF can be used as accessory cells.

The instant vehicle can also be used as a reservoir for the controlled delivery of needed drugs or biotherapeutics. In such cases, the core, rather than containing cells or tissues, contains a high c ncentration

of the selected drug or biotherapeutic. In addition, satellite vehicles containing substances which prepare or create a hospitable environment in the area of the body in which a biocompatible immunoisolatory vehicle containing isolated cells is implanted can also be implanted into a recipient. In such instances, the vehicle containing immunoisolated cells is implanted in the region along with satellite vehicles releasing controlled amounts of, for example, a substance which down-modulates or inhibits an inflammatory response from the recipient (e.g., anti-inflammatory steroids), or a substance which stimulates the ingrowth of capillary beds (i.e., an angiogenic factor).

The surrounding or peripheral region (jacket) of the instant vehicle is permselective, biocompatible, and immunoisolatory. It is produced in such a manner that it is free of isolated cells, and completely surrounds (i.e., isolates) the core, thereby preventing contact between any cells in the core and the recipient's body.

Turning first to the permselective nature of the jacket, it is formed in such a manner that it has a MWCO range appropriate both to the type and extent of immunological reaction it is anticipated will be encountered after the vehicle is implanted and to the molecular size of the largest substance whose passage into and out of the vehicle is desirable. The type and extent of immunological attacks which may be mounted by the recipient following implantation of the vehicle depend in part upon the type(s) of moiety isolated within it and in part upon the identity of the recipient (i.e., how closely the recipient is genetically related to the source of the biologically active moiety). When the implanted tissue is allogeneic to the recipient, immunological rejection may proceed largely through cell-mediated attack by the recipient's immune cells against the implanted cells.

When the tissue is xenog neic to the recipient, m lecular attack through assembly of the recipient's cytolytic complement attack complex may predominate, as well as the antibody interaction with complement.

The MWCO of the surrounding region must therefore be sufficiently low to prevent access of the substances required to carry out these attacks to the core, yet sufficiently high to allow delivery of the needed product to the recipient's body. It will therefore be apparent that the MWCO need not be strictly restricted to a range which excludes immunoglobulin G from the core. In fact, there are many cases in which higher MWCOs are not only permissible, but also advantageous. Indeed, higher MWCOs allow the delivery of a wide variety of useful products from immunoisolated cells, as well as the use of such cells to provide metabolic control of high molecular weight substances.

Thus, in appropriate cases, the peripheral or surrounding region can be made of materials which form permselective membranes or hydrogel matrices allowing the passage of molecules up to about the size of C1q (about 400 kD), the largest protein required for the assembly of the complement attack complex. Therefore, any cellular product or metabolite below about the size of C1q can pass freely through the vehicle. In other cases, it may still be desirable to exclude immunoglobulins. In such cases, materials which form matrices or membranes through which molecules which are equivalent to or larger than the size of immunoglobulin G (about 150 kD) cannot pass can be used. Cellular products or metabolites which are smaller than about 150 kD will still pass through the vehicle. In still other cases, where the patient is immunosuppressed or where the implanted tissue is syngeneic to the patient, a vigorous immunological attack is n t likely t be encountered, and passage f a high m lecular weight

m lecule may be desired. In these latter cases, materials which allow passage of all m lecules up to about the size of immunoglobulin M (about 1,000 kD) can be used. These materials will impede the passage of only very large substances, such as cells.

In another aspect of the invention, it has been found that a molecular weight cutoff for the jacket considerably higher than that previously contemplated may be employed while maintaining the viability and function of the encapsulated cells.

This permits the macrocapsules to be used in applications where the cells secrete a substance of high molecular weight. For this purpose, a macrocapsule with molecular cutoffs in excess of say 80 to 100 kD to as high as 200 to 1000 or 2000 kD or more may be employed in accordance with the present invention.

Turning next to the biocompatibility of the surrounding or peripheral region (jacket), this property is produced by a combination of factors. First, the materials used to form the vehicle are substances selected based upon their ability to be compatible with, and accepted by, the tissues of the recipient of the implanted vehicle. Substances are used which are not harmful to the recipient or to the isolated biologically active moiety. Preferred substances include reversibly and irreversibly gellable substances (e.g., those which form hydrogels), and water-insoluble thermoplastic polymers. Particularly preferred thermoplastic polymer substances are those which are modestly hydrophobic ,i.e. those having a solubility parameter as defined in Brandrup J., et al. (1989) Polymer Handbook 3rd Ed., John Wiley & Sons, NY, between 8 and 15, or more preferably, between 9 and 14 $(Joules/m^3)^{1/2}$. The polymer substances are chosen to have a solubility parameter low enough so that they are soluble in organic

solvents and still high enough so that th y will partition to form a proper membrane. Such polymer substances should be free of labile nucleophilic moieties and be highly resistant to oxidants and enzymes even in the absence of stabilizing agents. The period of residence *in vivo* which is contemplated for the particular immunoisolatory vehicle must also be considered: substances must be chosen which are adequately stable when exposed to physiological conditions and stresses. There are many hydrogels and thermoplastics which are sufficiently stable, even for extended periods of residence *in vivo*, such as periods in excess of one or two years. Examples of stable materials include alginate (hydrogel) and PAN/PVC (thermoplastic).

Second, substances used in preparing the biocompatible immunoisolatory vehicle are either free of leachable pyrogenic or otherwise harmful, irritating, or immunogenic substances or are exhaustively purified to remove such harmful substances. Thereafter, and throughout the manufacture and maintenance of the vehicle prior to implantation, great care is taken to prevent the adulturation or contamination of the vehicle with substances which would adversely affect its biocompatibility.

Third, the exterior configuration of the vehicle, including its texture, is formed in such a manner that it provides an optimal interface with the tissues of the recipient after implantation. This parameter will be defined in part by the site of implantation. For example, if the vehicle will reside in the peritoneal cavity of the recipient, its surface should be smooth. However, if it will be embedded in the soft tissues of the recipient, its surface can be moderately rough or stippled. A determining factor will be whether it is desirable to allow cells of the recipient to attach to the external surface of the vehicle r if such attachment must be

avoided. An open-textured or sponge-like surface may promote the ingrowth of capillary beds, whereas a smooth surface may disc urage excessive overgr wth by fibroblasts. Excessive overgrowth by fibroblasts is to be avoided, except where capillary undergrowth has occurred, as it may result in the deposition of a poorly-permeable basement membrane around the vehicle and walling off of the isolated cells from contact with the recipient's body.

Certain vehicle geometries have also been found to specifically elicit foreign body fibrotic responses and should be avoided. Thus vehicles should not contain structures having interlayers such as brush surfaces or folds. In general, opposing vehicle surfaces or edges either from the same or adjacent vehicles should be at least 1 mm apart, preferably greater than 2 mm and most preferably greater than 5 mm. Preferred embodiments include cylinders, "U"-shaped cylinders, and flat sheets or sandwiches.

The surrounding or peripheral region (jacket) of the biocompatible immunoisolatory vehicle can optionally include substances which decrease or deter local inflammatory response to the implanted vehicle, and/or generate or foster a suitable local environment for the implanted cells or tissues. For example antibodies to one or more mediators of the immune response could be included. Available potentially useful antibodies such as antibodies to the lymphokines tumor necrosis factor (TNF), and interferon (IFN) can be included in the matrix precursor solution. Similarly, an anti-inflammatory steroid can be included. Christenson, L., et al., (1989) J. Biomed. Mat. Res. (23):705-718; Christenson, L., 1989, Ph.D. thesis, Brown University, incorporated by reference. Alternatively, a substance which stimulates angiogenesis (ingrowth of capillary beds) can be included; this may be particularly desirable where the isolated cells or tissues

require close contact with the r cipient's bl odstream to function properly (e.g., insulin-producing islets of Langerhans). Cell which are genetically engineered to secrete antibodies may also be included in the matrix.

Turning now to the concept of immunoisolation, the surrounding or peripheral region further confers protection of the biologically active moiety from the immune system of the individual in whom the vehicle is implanted by preventing harmful substances of the individual's body from entering the core of the vehicle, and by providing a physical barrier sufficient to prevent detrimental immunological contact between the isolated moiety and the individual's immune system. The thickness of this physical barrier can vary, but it will always be sufficiently thick to prevent direct contact between the cells and/or substances on either side of the barrier. The thickness of this region generally ranges between 5 and 200 microns; thicknesses of 10 to 100 microns are preferred, and thickness of 20 to 50 microns are particularly preferred. Types of immunological attack which can be prevented or minimized by the use of the instant vehicle include attack by macrophages, neutrophils, cellular immune responses (e.g. natural killer cells and antibody-dependent T cell-mediated cytoloysis [ADCC]), and humoral response (e.g. antibody-dependent complement mediated cytolysis).

As discussed above, the type and extent of immunological response by the recipient to the implanted vehicle will be influenced by the relationship of the recipient to the isolated biologically active moiety. For example, if the isolated materials comprise syngeneic cells, these will not cause a vigorous immunological reaction, unless the recipient suffers from an autoimmunity with respect to the particular cell or tissue type within the vehicle. There are several disease or deficiency states which have

recently be n determined to have an aut immun eti logy, most notably Type I, insulin-dependent Diabetes mellitus, wh rein the insulin secreting pancreatic islet β cells are destroyed by the individual's immune system. Fan, M.-Y., et al., Diabetes, 39: 519-522 (1990).

Syngeneic cells or tissue are rarely available. In many cases, allogeneic or xenogeneic cells or tissue (i.e., from donors of the same species as, or from a different species than, the prospective recipient) may be available. The immunoisolatory vehicle allows the implantation of such cells or tissue, without a concomitant need to immunosuppress the recipient. Therefore, the instant vehicle makes it possible to treat many more individuals than can be treated by conventional transplantation techniques. For example, far more patients suffer from Type 1 diabetes than can be transplanted with human donor islets (in 1990, fewer than about 4,000 suitable cadaver organ donors became available in the U.S. for all organ transplants). The supply of donor porcine or bovine islets is far greater; if these xenoislets are appropriately immunoisolated according to the instant invention, the diabetic condition of a far greater number of patients can be remedied. The type and vigor of an immune response to xenografted tissue is expected to differ from the response encountered when syngeneic or allogeneic tissue is implanted into a recipient. This rejection may proceed primarily by cell-mediated, or by complement-mediated attack; the determining parameters in a particular case may be poorly understood. However, as noted previously, the exclusion of IgG from the core of the vehicle is not the touchstone of immunoprotection, because IgG alone is insufficient to produce cytolysis of the target cells or tissues. Using the macrocapsules of the present invention, preferably with allogeneic tissue, but even with xenografts, it is possible to deliver needed high molecular weight products or to provide metabolic

functions pertaining to high molecular weight substances, provided that critical substances necessary to the mediation of immunological attack are excluded from the immunoisolatory vehicle. These substances may comprise the complement attack complex component C1q, or they may comprise phagocytic or cytotoxic cells; the instant immunoisolatory vehicle provides a protective barrier between these harmful substances and the isolated cells. Thus, the present immunoisolatory vehicle can be used for the delivery even from allogeneic or xenogeneic cells or tissue, products having a wide range of molecular sizes, such as insulin, parathyroid hormone, interleukin 3, erythropoietin, albumin, transferrin, and Factor VIII.

In another embodiment of this invention, methods are provided for the treatment of diseases caused by neural degeneration. Examples of human diseases which are thought to be associated with neural degeneration include Alzheimer's disease, Huntington's chorea, AIDS-related dementia, and Parkinson's disease.

Animal models for neurodegenerative conditions are based on the premise that a specific insult may damage or kill neurons. In some cases this may even lead to a cascade of neuronal death which affects trophically interdependent neurons along pathways responsible for specific brain functions.

A strategy for treatment of neural degenerative condition involves the localized administration of growth or trophic factors in order to (1) inhibit further damage to post-synaptic neurons, and (2) improve viability of cells subjected to the insult. Factors known to improve neuronal viability include basic fibroblast growth factor, ciliary neurotrophic factor, brain-derived neurotrophic factor, neurotrophin-3, neurotensin, and Substance P.

In one animal model for neurodegenerative excitotoxicity, the glutamate analog, quinolinic acid, is injected stereotaxically into the brain region known as the striatum and/or basal ganglia to produce neuropathology and symptoms analogous to those of patients suffering from Huntington's disease. Both the model and actual Huntington's disease are characterized by damage to neurons necessary for aspects of motor control.

Furthermore, one of the early symptoms of Huntington's disease is loss of body weight (Sanberg, P.R. et al. 1981 Med J Aust. 1, 407-409. Similar effects are also seen in the model system (Sanberg, P.R. et al. 1979 Exp Neurol 66, 444-466. Quinolinic acid is also found at abnormally high levels in AIDS-related dementia.

According to the present invention, trophic factors are provided to the proper brain region by implanting a vehicle containing living cells which secret an appropriate factor. Suitably, the cells are adrenal chromaffin cells which are known to secrete at least one factor, basic fibroblast growth factor. Other as yet unidentified trophic factors may also be secreted by chromaffin cells. It is to be noted that this embodiment of the invention is separate from the use of chromaffin cells to secrete the neurotransmitter, dopamine, for the amelioration of symptoms of Parkinson's disease. Nerve growth factor-secreting cells such as fibroblasts engineered to express NGF represent an alternative therapy for this quinolinic acid induced neurodegeneration. Schwann cells prepared from myelin may be encapsulated and implanted in appropriate brain areas to prevent neural degeneration associated with Parkinson's disease

In another embodiment of the invention, the animal model involves lesion of the fimbria-fornix. In particular, neurons of the septohippocampal system are axotomized

which leads to degeneration and cell death. This model is thought to be analogous to the types of lesions which cause Alzheimer's disease in humans. Suitably, a growth factor, nerve growth factor (NGF), is provided by the implantation of a vehicle containing cells which secrete NGF. Astrocytes immortalized (e.g. by transformation with the Large T antigen) and genetically engineered to express NGF may be employed. Preferably, the cells are fibroblasts which have been genetically engineered to produce recombinant NGF. The fibroblasts survive best in a core composed of a matrix material which mimicks extracellular matrix, such as collagen or laminin-containing hydrogels. The core is surrounded by an immunoisolatory jacket which allows the diffusion of oxygen and nutrients to the cells in the core, and also allows the secreted NGF to diffuse through the jacket and into the body of the recipient. The vehicle implant inhibits the death of cholinergic neurons as assayed by the number of neurons which contain choline acetyl transferase (ChAT), an indicator of viable cholinergic neurons.

Fimbria-fornix lesions also cause behavioral deficits in the animal subjects of the model, most easily observed in tasks involving learning and memory. It has been reported that chronic administration of NGF to rats with fimbria-fornix lesions accelerates the animals' behavioral recovery (Wills, B. et al. 1985 Behav.Brain Res. 17, 17-24). In the present invention, implantation of the vehicle containing NGF-secreting cells provides a practical way to deliver NGF continuously to the appropriate brain region of the lesioned animal. By analogy, the vehicle of the present invention offers a practical form of regenerative and/or prophylactic therapy for Alzheimer's victims whose conditions may be ameliorated by continuous delivery of NGF t specific brain regions.

The present immunoisolatory vehicle can be formed in a wide variety of shapes and combinations of suitable materials. A primary consideration in selecting a particular configuration for the vehicle when cells are present is the access of oxygen and nutrients to the isolated cells or tissues, and passage of waste metabolites, toxins and the secreted product from the vehicle. The immunoisolatory vehicle can be any configuration appropriate for maintaining biological activity and providing access for delivery of the product or function, including for example, cylindrical, rectangular, disk-shaped, patch-shaped, ovoid, stellate, or spherical. Moreover, the vehicle can be coiled or wrapped into a mesh-like or nested structure. If the vehicle is to be retrieved after it is implanted, configurations which tend to lead to migration of the vehicle(s) from the site of implantation, such as spherical vehicles small enough to travel in the recipient's blood vessels, are not preferred. Certain shapes, such as rectangles, patches, disks, cylinders, and flat sheets offer greater structural integrity and are preferable where retrieval is desired.

The instant vehicle must provide, in at least one dimension, sufficiently close proximity of any isolated cells in the core to the surrounding tissues of the recipient, including the recipient's bloodstream, in order to maintain the viability and function of the isolated cells. However, the diffusional limitations of the materials used to form the vehicle do not in all cases solely prescribe its configurational limits. Certain additives can be used which alter or enhance the diffusional properties, or nutrient or oxygen transport properties, of the basic vehicle. For example, the internal medium can be supplemented with oxygen-saturated perfluorocarbons, thus reducing the needs for immediate contact with blood-borne oxygen. This will allow isolated

cells or tissues to remain viable while, f r instance, a gradient of angiotensin is released from the vehicle into the surrounding tissues, stimulating ingrowth of capillaries. References and methods for use of perfluorocarbons are given by Faithful, N.S. (1987) Anaesthesia 42: 234-242 and NASA Tech Briefs MSC-21480, U.S. Govt. Printing Office, Washington, D.C. 20402, incorporated herein by reference. Alternatively for clonal cell lines such as PC12 cells, genetically engineered hemoglobin sequences may be introduced into the cell lines to produce superior oxygen storage. Vol. 15 #1 page 54. NPO-17517 NASA Tech Briefs.

In general, in the absence of oxygen carrier additives, when the cells are present the vehicle will have a maximum depth-to-surface distance of no more than 2 mm in at least one dimension, with a maximum depth of 800 microns being preferred. One or several vehicles may be required to produce the desired effect in the recipient.

The thickness of the immunoisolatory vehicle jacket should be sufficient to prevent an immunoresponse by the patient to the presence of the vehicles. For that purpose, the vehicles preferably have a minimum thickness of 1μm or more free of the cells.

Additionally, reinforcing structural elements can be incorporated into the vehicle. These structural elements can be made in such a fashion that they are impermeable, and are appropriately configured to allow tethering or suturing of the vehicle to the tissues of the recipient. In certain circumstances, these elements can act to securely seal the surrounding or peripheral region (e.g., at the ends of a cylindrical vehicle, or at the edges of a disk-shaped vehicle), completing isolation of the core materials (e.g., a molded thermoplastic clip). For many c nfigurations, it is desirable that these structural

elements sh uld not occlude a significant area of th permselective surrounding or peripheral region.

In one preferred embodiment, the implantabl immunoisolatory vehicle of the present invention is of a sufficient size and durability for complete retrieval after implantation. To be contrasted with such microcapsules, which have a typical maximum practical volume on the order of 1μl, the preferred immunoisolatory vehicle of the present invention is termed "macrocapsule". Such macrocapsules have a core of a preferable minimum volume of about 1 to 10μl and depending upon use are easily fabricated to have a value in excess of 100μl.

In terms of retrievability, microspheres are generally less practical than macro-capsules. In order for tissue encapsulated in microspheres to provide a therapeutic dose of insulin, for instance, the number of microspheres must be increased to such a large extent that significant retrievability becomes impossible. Additionally, an increase in the volume of tissue placed within a microsphere requires a corresponding increase in surface area. Within a sphere, because surface area scales with r^2 where as volume scales with r^3, as the volume of encapsulated tissue volume increases, the required capsule size to provide sufficient surface area for nutrient diffusion to the encapsulated tissue quickly becomes impractical.

Macrocapsules in the shapes of cylinders or flat sheets do not have these limitations because volume increases more proportionately to surface area such that the diffusional transport of nutrients and products for increased amounts of tissue can be accommodated by increasing the surface area without unweidly increases in total vehicle size. If, for example, about 10,000 islets are required per kg body weight to restore normoglycemia to a diabetic

patient, from 1,000 to 10,000 microcapsules must be transplanted per kg body weight (e.g. 1-10 islets per capsule). This number of microcapsules could not be easily retrieved, if retrieval were required. In contrast, the macrocapsules of the instant invention may easily hold greater than 1,000 islets to as high as 500,000 islets or more per vehicle. The preferred embodiment would require fewer than 5-10 vehicles per patient, making macrocapsules more easily retrieved than a large number of microcapsules.

The macrocapsules of the present invention are distinguished from microcapsules (Sun, A.M., supra; Rha, C-K., U.S. Patent 4,744,933) by the capacity of macrocapsules to contain over 10^4 cells and maintain them in viable condition. The droplet methods used in the making of microcapsules, in order to ensure cell viability, necessarily limit the number of cells per capsule to fewer than 10^4.

The instant invention also relates to a method for making an immunoisolatory vehicle. As noted previously, the vehicle can provide for the implantation of diverse cell or tissue types, including fully-differentiated, anchorage-dependent, fetal or neonatal, or transformed, anchorage-independent cells or tissue. The cells to be immunoisolated are prepared either from a donor (i.e., primary cells or tissues, including adult, neonatal, and fetal cells or tissues) or from cells which replicate *in vitro* (i.e., immortalized cells or cell lines, including genetically modified cells). In all cases, a sufficient quantity of cells to produce effective levels of the needed product or to supply an effective level of the needed metabolic function is prepared, generally under sterile conditions, and maintained appropriately (e.g. in a balanced salt solution such as Hank's salts, r in a nutri nt medium, such as Ham's F12) pri r to isolation.

In another aspect of the invention, the macrocapsules are of a shape which tends to reduce the distance between the center of the macrocapsule and the nearest portion of the jacket for purposes of permitting easy access of nutrients from the patient into the cell or of entry of the patient's proteins into the cell to be acted upon by the cell to provide a metabolic function, such as interaction with cholesterol or the like. In that regard, a non-spherical shape is preferred, such as a long tube or flat plate, or the like. The optimum shape for this purpose may be calculated by known techniques as set forth herein.

Four important factors influencing the number of cells or amount of tissue to be placed within the core of the biocompatible immunoisolatory vehicle (i.e., loading density) of the instant invention are: (1) vehicle size and geometry; (2) mitotic activity within the vehicle; (3) viscosity requirements for core preparation and or loading; and (4) pre-implantation assay and qualification requirements.

With respect to the first of these factors, (capsule size and geometry), the diffusion of critical nutrients and metabolic requirements into the cells as well as diffusion of waste products away from the cell are critical to the continued viability of the cells. Since diffusional access to the contents of the vehicle is limited by vehicle surface area, surface to volume relationships of various shapes and size vehicles will be critical in determining how much viable tissue can be maintained within the vehicle. Among the metabolic requirements met by diffusion of substances into the vehicle is the requirement for oxygen. The oxygen requirements of the specific cells must be determined for the cell of choice. Methods and references for determination of oxygen metabolism are given in Wilson D.F. et al., (1988) J. Biol. Chem. 263:2712-2718. The oxygen requirement for

islet cells has been applied to coupled diffusi n r acti n models accounting for diffusional transport from surrounding tissue through the vehicle wall and tissue compartment (core), and used to calculate the expected viability of islet cells in a number of vehicles of different sizes and configurations, after the method of Dionne, K.E., (1989), Thesis Ph.D., Massachusetts Institute of Technology. For intact pancreatic islets, these calculations agree well with experimental observations. For a cylindrical vehicle of 900 microns outer diameter, implanted into the peritoneal cavity ($pO_2 \approx$ 45-50 mmHg), the optimal total cell volume is in the range of up to 20%, preferably 1 - 15%, most preferably about 5% of the vehicle volume. (If this capsule were 20cm in length it would have a volume of 100mm^3. To provide the same amount of surface area with a single sphere, e.g. to support comparable amounts of tissue, would require a volume of 1,047mm^3. For a cylindrical vehicle of 400 microns the optimal cell volume is between 35-65% total vehicle volume, and is preferably 35%. These calculations also take into account the partial oxygen pressure at the site of implantation. At implantation sites where the oxygen pressure is less than the peritoneum (e.g., subcutaneous $pO_2 \approx 20$), lower loading densities will be required. Implantation into arteries (pO_2 95mmHg) and the brain (pO_2>75 mmHg) will allow support of greater tissue volume per unit vehicle. Other vehicle configurations, such as disk-shaped or spherical, are also possible and optimal cell volumes may be similarly calculated for these geometries. Actual loading densities will consider not only these diffusional considerations but also the other considerations given below.

With respect to the second factor (cell division), if the cells selected are expected to be actively dividing while in the vehicle, then they will continue to divide until they fill the available space, or until phenom na such as

contact inhibition limit further division. F r replicating cells, the geometry and size of the vehicle will be chosen so that complete filling of the vehicle core will not lead to deprivation of critical nutrients due to diffusional limitations. In general, vehicles that will be filled to confluency with cells or tissue will be no more than 250 microns in cross-section, such that cells in the interior will have less than 15 cells between them and an external diffusional surface, preferably less than 10 cells and more preferably less than 5 cells.

In general, for cells not expected to divide within the vehicle, such as chromaffin cells, pancreatic islet cells and the like, the appropriate cell densitites will be calculated from the diffusional considerations listed above.

With respect to the third factor (viscosity of core materials) cells in densities occupying up to 70% of the vehicle volume can be viable, but cell solutions in this concentration range would have considerable viscosity. Introduction of cells in a very viscous solution into the vehicle could be prohibitively difficult. In general, for both two step and coextrusion strategies, discussed below, cell loading densities of higher than 30% will seldom be useful, and in general optimal loading densities will be 20% and below. For fragments of tissues, it is important, in order to preserve the viability of interior cells, to observe the same general guidelines as above and tissue fragments should not exceed 250 microns in diameter with the interior cells having less than 15, preferably less than 10 cells between them and the nearest diffusional surface.

Finally, with respect to the fourth factor (preimplantation and assay requirements), in many cases, a certain amount of time will be required between vehicle

can interfere with th efficacy f the implant. Vehicles of the instant inventi n can easily be manufactured which c ntain many thousands f cells. In preferr d embodiments, therapeutically useful immunoisolatory vehicles used to provide insulin to insulin deficient rats contained on the order of 1,000 islets. Larger vehicles can also be conveniently prepared by the method of the current invention. Because of the potentially large capacity of the immunoisolatory vehicles, the treatment of many conditions will require only one or at most a few (less than 10) implanted vehicles to supply an appropriate therapeutic dose. The use of only a few therapeutically effective implantable vehicles containing a large number of cells provides simple retrievability which, for many applications, will be preferred over microsphere or other small configurations requiring a large number of vehicles. The immunoisolatory macrocapsule of the present invention is capable of storing 10,000 to 100,000 cells to as high as 500,000 cells or more, in individual or cluster form, depending on their type.

Techniques and procedures for isolating cells or tissues which produce a selected product are known to those skilled in the art, or can be adapted from known procedures with no more than routine experimentation. For example, islets of Langerhans can be isolated from a large-animal pancreas (e.g., human or porcine) using a combination of mechanical distention and collagenase digestion, as described by Scharp, D.W., et al., (1989) in U.S. Patent No. 4,868,121. Islets may be isolated from small animals such as rats by the method of Scharp, et al., (1980) Diabetes 29:suppl 1, 19-30. Similarly, hepatocytes can be isolated from liver tissue using collagenase digestion followed by tissue fractionation, as described by Sun, A.M., et al., (1987) Biomat., Art. Cells, Art. Org. 15(2):483-496. Adrenal Chromaffin cells may be isolated by the method of Livett, B.G. (1984)

Physiology Reviews 64:1103-1161. Many cellular products which are difficult to provide using primary donor tissues can be provided using immortalized cells or cell lines. Immortalized cells are those which are capable of indefinite replication but which exhibit contact inhibition upon confluence and are not tumorigenic. An example of an immortalized cell line is the rat pheochromocytoma cell line PC12. Transformed cells or cell lines can be used in a similar manner. Transformed cells are unlike merely immortalized cells in that they do not exhibit contact inhibition upon confluence, and form tumors when implanted into an allogeneic host. Immortalization can allow the use of rare or notoriously fragile cell or tissue types for the long-term delivery of a chosen product or metabolic function. Suitable techniques for the immortalization of cells are described in Land H. et al. (1983) Nature 304:596-602 and Cepko, C.L., (1988) Neuron 1:345-353. Candidate cell lines include genetically engineered beta-cell lines which secrete insulin such as NIT cells (Hamaguchi, K., et al., 1991 Diabetes 40:842), RIN cells (Chick, W.L., et al., PNAS 1977 74:628-632), ATT cells (Hughes, S.D., et al, 1992, PNAS USA 89:688-692), CHO cells (Matsumoto, M., et al, 1990, PNAS USA 87:9133-9137), and beta-TC-3 cells (Tal, M., et al, 1992, Molec. and Cell Biol. 12:422-432). Additionally, recombinant cells or cell lines can be engineered to provide novel products or functions and combinations thereof, using a wide variety of techniques well known to those of ordinary skill in the art.

For example, fibroblasts can be transfected with an expression vector for the chosen product (e.g., nerve growth factor, erythropoietin, insulin, or Factor VIII). It should be recognized however, that expression of a recombinant protein in a cell type which does not normally express the protein may lead to unregulated expression

which may not be desirable for certain medical applications.

B-cell hybridomas secreting a selected monoclonal antibody, or T-cell hybridomas secreting a selected lymphokine, can also be used. It may be particularly desirable to deliver a monoclonal antibody or fraction thereof, which neutralizes the biological activity of a disregulated biological response modifier using the instant invention. Engineered cells which secrete soluble fragments of receptors for these biological response modifiers can be used in a similar fashion. The disregulation or overproduction of particular biological response modifiers has been implicated in the etiology of certain cancers.

If the cells to be immunoisolated are replicating cells or cell lines adapted to growth in vitro, it is particularly advantageous to generate a cell bank of these cells. A particular advantage of a cell bank is that it is a source of cells prepared from the same culture or batch of cells. That is, all cells originated from the same source of cells and have been exposed to the same conditions and stresses. Therefore, the vials can be treated as identical clones. In the transplantation context, this greatly facilitates the production of identical or replacement immunoisolatory vehicles. It also allows simplified testing protocols which assure that implanted cells are free of retroviruses and the like. It may also allow for parallel monitoring of vehicles *in vivo* and *in vitro*, thus allowing investigation of effects or factors unique to residence *in vivo*.

In all cases, it is important that the cells or tissue contained in the vehicle are not contaminated or adulterated. If a vehicle having a matrix core is desired, the cells are mixed under sterile conditions,

with an appropriate amount of a bi compatible, gellable hydrogel matrix precursor. There are numerous natural and synthetic hydrogels which are suitable for use in a biocompatible immunoisolatory vehicle of the instant invention. Suitable naturally-derived hydrogels include plant-derived gums, such as the alkali metal alginates and agarose, and other plant-derived substances, such as cellulose and its derivatives (e.g., methylcellulose). Animal tissue-derived hydrogels such as gelatin are also useful. Alternatively, the core matrix can be made of extracellular matrix (ECM) components, as described by Kleinman et al., (1989) U.S. Patent No. 4,829,000. Suitable synthetic hydrogels include polyvinyl alcohol, block copolymer of ethylene-vinylalcohol, sodium polystyrene sulfonate, vinyl-methyl-tribenzyl ammonium chloride and polyphosphazene (Cohen, S. et al. 1990 J. Anal. Chem. Soc. 112:7832-7833).

If a dual matrix immunoisolatory vehicle is to be formed, the surrounding or peripheral region can be made of a hydrogel selected from the above-listed matrix precursors. If the surrounding or peripheral region of the vehicle is to comprise a permselective membrane, other precursor materials can be used. For example, the surrounding or peripheral region can be made from water-insoluble, biocompatible thermoplastic polymers or copolymers. Several of the polymers or copolymers taught by Michaels (1971) U.S. Patent No. 3,615,024, which is hereby incorporated by reference, fulfill these criteria. A preferred membrane casting solution comprises a polyacrylonitrile-polyvinylchloride (PAN/PVC) copolymer dissolved in the water-miscible solvent dimethylsulfoxide (DMSO). This casting solution can optionally comprise hydrophilic or hydrophobic additives which affect the permeability characteristics of the finished membrane. A preferred hydrophilic additive for the PAN/PVC c polymer is polyvinylpyrrolidone (PVP). Other suitable polymers

comprise polyacryl nitrile (PAN), p lymethylmethacrylate (PMMA), polyvinyldifluoride (PVDF), polyethylene oxide, polyolefins (e.g., polyisobutylene or polypropylene), polysulfones, and cellulose derivatives (e.g., cellulose acetate or cellulose butyrate). Compatible water-miscible solvents for these and other suitable polymers and copolymers are found in the teachings of U.S. Patent No. 3,615,024.

In a preferred embodiment, the core is surrounded by a biocompatible hydrogel matrix free of cells projecting externally from the outer layer. The macrocapsules of the present invention are distinguished from the microcapsules of Rha, Lim, and Sun (Rha, C.K. et al, U.S. Patent 4,744,933; Sun, A.W., supra) by (1) the complete exclusion of cells from the outer layer of the macrocapsule, and (2) the thickness of the outer layer of the macrocapsule. Both qualities contribute to the immunoisolation of encapsulated cells in the present invention. The microcapsules of Rha were formed by ionic interaction of an ionic core solution with an ionic polymer of opposite charge. The microcapsules of Lim and Sun were formed by linking an external hydrogel jacket to the core through an intermediate layer of poly-L-lysine (PLL).

In the microcapsules of Lim and Sun, the intermediate PLL layer was not sufficiently thick to guarantee that portions of the encapsulated cells would not penetrate through and beyond the layer. Cells penetrating the PLL layer are potential targets for an immune response. All these capsules, including those disclosed by Rha, also suffer the following additional limitations: (a) they are round, and (b) the formation of the outer layer is dependent upon direct ionic bonding or polyamide linkage with an inner layer or core substance. The disadvantages

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1	LET.	1
2	XT/	1
3	AF/D	2
4	A...	1
5	CLM	2
6	REM	3

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